Transmission of Vesicular Stomatitis New Jersey Virus to Cattle by the Biting Midge *Culicoides sonorensis* (Diptera: Ceratopogonidae)

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ABSTRACT Laboratory-reared *Culicoides sonorensis* Wirth & Jones were infected with vesicular stomatitis virus serotype New Jersey (family *Rhabdoviridae*, genus *Vesiculovirus*, VSNJV) through intrathoracic inoculation. After 10-d incubation at 25°C, these insects were allowed to blood feed on four steers. Two other steers were exposed to VSNJV through intralingual inoculation with 10^8 tissue culture infective $dose_{50}$ VSNJV. All six steers became seropositive for VSNJV. The results demonstrate the ability of *C. sonorensis* to transmit VSNJV to livestock. Only the animals intralingually inoculated with VSNJV showed clinical signs in the form of vesicles at the site of inoculation. Uninfected *C. sonorensis* allowed to feed on the exposed animals did not become infected with VSNJV. Animals infected by *C. sonorensis* showed a slower antibody response compared with intralingually inoculated animals. This is probably because of different amounts of virus received via insect transmission and syringe inoculation. A significant difference was found in the serum acute-phase protein α-1-acid glycoprotein in animals that received VSNJV through *C. sonorensis* transmission. These animals had previously been exposed to insect attack in the field compared with intralingually inoculated animals and *C. sonorensis*-infected animals that had been protected from insect attack. The failure to observe clinical signs of vesicular stomatitis through transmission of VSNJV by *C. sonorensis* may explain widespread subclinical infections during vesicular stomatitis epidemics.

KEY WORDS *Culicoides*, vector competence, vesicular stomatitis, cattle

EPIDEMICS OF VESICULAR STOMATITIS virus (family *Rhadoviridae*, genus *Vesiculovirus*, VSV) occur periodically among livestock in the western United States. In addition to the financial impact from clinically affected animals, vesicular stomatitis outbreaks can cause economic losses for agriculture because of quarantining of affected premises and regional, national, and international restrictions on the trade of U.S. livestock (Hayek et al. 1998). Vesicular stomatitis is a reportable disease and requires prompt diagnosis because it is clinically indistinguishable from clinical signs produced by foot-and-mouth disease virus (family *Picornaviridae*, genus *Aphthovirus*, FMDV) among cloven-hoofed animals. Disease surveillance based on clinical observations can be misleading because only a small proportion of infected animals develop clinical signs (Tesh 1994, Hayek et al. 1998), and <30% of lingual lesions in cattle may develop into grossly evident vesicles (Seibold and Sharp 1960). Investigations of the mechanisms determining the epidemiology of VSV in the western United States are essential for the development of efficient prevention and control methods.

Domestic animals are not considered as amplifying hosts of VSV for insect vectors because of a low and transient viremia that is developed upon infection (Tesh 1994, Comer et al. 1995). It has been suggested that high viral titers in skin associated with vesicular lesions may be a source of the virus for nonsystemic transmission by insect vectors (Stallknecht et al. 1993). Nonsystemic transmission of VSV to blackflies *Simulium vittatum* Zetterstedt has been demonstrated (Mead et al. 2000). Several studies have demonstrated that *S. vittatum* can be infected and transmit the virus to livestock in the United States (Mead et al. 1999, 2000, 2004a,b). Although several blood-feeding insect species are suspected vectors of VSV, their roles during VSV epidemics in the western United States remain unclear.

The biting midge *Culicoides sonorensis* Wirth & Jones is indigenous to the western United States, and VSV serotype New Jersey (family *Rhabdoviridae*, genus *Vesiculovirus*, VSNJV) was isolated from individuals of this species during vesicular stomatitis outbreaks in 1982–1983 (Walton et al. 1987, Kramer et al.

Procedures for animal care and use were approved by the Institutional Animal Care and Use Committee of the University of Wyoming where the animal experiments were performed. The investigators adhered to the Guide for the Care and Use of Laboratory Animals as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

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1990). *C. sonorensis* can become infected with VSNJV by feeding on infected blood through an artificial membrane in the laboratory (Nunamaker et al. 2000). Additionally, VSNJV virus can be found in a variety of *C. sonorensis* tissues (Drolet et al. 2005), and infected *C. sonorensis* transmitted VSNJV to guinea pigs through blood feeding (Pérez de León et al. 2006). In this report, we compare the responses to VSNJV between cattle receiving the virus by lingual syringe inoculation or through the bite of infected *C. sonorensis.* The implications of VSV transmission by blood-feeding insects during epidemics in the western United States are discussed.

Materials and Methods

Virus. The strain 8/82-CO of VSNJV isolated from *C. sonorensis* during the 1982–1983 epidemic in Colorado (Walton et al. 1987) was used to infect the insects and steers. This strain of VSNJV had been shown to produce vesicles after inoculation in the snout of swine (Redelman et al. 1989) and the footpads of guinea pigs (Pérez de León et al. 2006). Stock virus was passaged once in African green monkey kidney (Vero) cells. Virus suspensions containing 10^6 or 10^8 median tissue culture infective doses ($TCID_{50}$)/ml were used as indicated.

Animals. Seven steers, *Bos taurus* L., weighing 156–220 kg were housed under Biosafety level (BL)-3 biocontainment with free access to water and hay pellets. Six steers, all but 158, had been used before the insect transmission studies reported here to characterize the effect of biting fly attack on cattle physiology (W.J.T., unpublished data). Three (68, 515, and 540) of the six steers were exposed to biting flies in the field, whereas the others (183, 220, and 376) were protected from biting fly attack. The insect-exposed steers were each attacked by as many as 100,000 biting flies representing several species of mosquitoes and blackflies during a 4-wk period based on insect trapping from the animals during this period (unpublished data).

Insects. *C. sonorensis* were from a laboratory colony designated AA (Jones 1960). The insects were reared to the adult stage and maintained on a 10% sucrose solution before use. Intrathoracic inoculations of 2-d-old adult biting midges with a suspension of VSNJV containing 10^6 $TCID_{50}$/ml were administered inside a glove box under BL-3 containment (Hunt and Tabachnick 1996). Intrathoracically inoculated biting midges were held in an incubator for a minimum of 10 d at 20°C, 70% RH, and a photoperiod of 13:11 (L:D) h, with free access to sugar water. Uninoculated 2-d-old insects or intrathoracically inoculated insects were placed in cylindrical plastic feeding cages and allowed to take a bloodmeal from the clipped flank or top butt of steers for 30 min. Intrathoracically inoculated insects were stored at −70°C after blood feeding. Uninoculated insects that blood fed on steers were frozen after incubation for at least 10 d as described above.

Experimental Design. Each of three pairs of steers was kept in a separate BL-3 animal isolation room. A steer previously exposed in the field to feeding by biting flies was randomly paired with a steer that had been protected from biting fly attack during the same period. A seventh steer (158), which had been protected from exposure to biting fly attack in the field, was used as negative control and housed individually in a fourth BL-3 isolation room.

After sedation with xylazine (0.05 mg/kg) applied intramuscularly, steers 68 and 183 were inoculated intradermally in the tongue with 1 ml of a suspension of VSNJV containing 10^8 $TCID_{50}$/ml. Each of four sites in the tongue received ≈250 μl of the viral inoculum. A negative control steer was treated similarly except that it was inoculated with only the VSNJV culture medium (Eagle's minimal essential medium containing 10% fetal bovine serum, 200 U/ml penicillin, 200 μg/ml streptomycin, 100 μg/ml gentamycin, 100 μg/ml neomycin, and 5 μg/ml amphotericin B). Steers in the other two pairs were infected with VSNJV by exposing them to feeding by intrathoracically inoculated biting midges.

Because the presence of a transient viremia in cattle has been reported previously (Hanson and Brandly 1957), the potential ability of cattle to serve as a source of VSNJV infection for blood-feeding insects was tested by allowing unfed and uninoculated adult biting midges to feed on the six infected steers. Different cohorts of uninfected biting midges were fed on steers infected by intralingual inoculation, or after exposure to inoculated midges. Uninfected biting midges were fed on steers 220 and 515 at 24 h, and steers 376 and 540 at 48 h, after the steers were exposed to intrathoracically inoculated biting midges (Table 1).

The steers were inspected for clinical signs of vesicular stomatitis and their rectal temperatures recorded twice daily for 10 d after infection. Attention was paid to the development of any grossly evident vesicles in the tongue (Seibold and Sharp 1960). Oral swabs were collected daily in tubes containing 1 ml of culture medium throughout the experiment. Serum samples were obtained before infection and daily throughout the experiment and stored at −20°C until used. Steers were euthanized at 40 d postinfection by an overdose of pentobarbital applied intravenously, and then necropsied.

Virus Assays. Frozen insects were individually homogenized in 100 μl of culture medium. Insect homogenates were clarified by centrifugation for 1 min at 14,000 rpm in a microcentrifuge, and 30 μl of the supernatant was diluted 1:10 with culture medium. The diluted supernatants were assayed in triplicate by adding 50 μl per well in a microplate containing Vero cells. Cell monolayers were inspected for cytopathic effect (CPE) after 3 d postinoculation to assess infection of individual biting midges. Homogenates of non-blood-fed uninoculated biting midges did not cause CPE in Vero cell monolayers.

Tubes with oral swabs were vortexed vigorously and the medium assayed for infectivity as described for the diluted supernatants of insect homogenates. The presence of VSNJV in samples causing CPE in Vero cell monolayers was confirmed by the fluorescent anti-

Table 1. Exposure of infected cattle to blood feeding by intrathoracically inoculated or host naive intact midges

Steer no.	Exposed to inoculated biting midges				Exposed to uninfected midges 24 h postinfection				Exposed to uninfected midges 48 h postinfection			
	BF	NBF	Total	%	BF	NBF	Total	%	BF	NBF	Total	%
68	—[a]				60	100	160	37.5	166	281	447	37.1
183	—				277	240	517	53.6	318	66	384	82.8
220	78	214	292	26.7	180	167	347	51.9	NA	NA	NA	NA
515	239	178	417	57.3	6	320	326	0.02	NA	NA	NA	NA
376	42	270	312	13.5	NA	NA	NA	NA	245	176	421	58.2
540	190	256	446	42.6	NA	NA	NA	NA	90	195	285	31.6

BF, number of blood-fed biting midges; NA, not applicable; NBF, nonblood-fed midges.
[a] Infected by lingual injection with 10^8 $TCID_{50}$ of VSNJV.

body cell culture technique using a fluorescein isothiocyanate-antibody conjugate (Snyder et al. 1982).

Serologic Tests. Serum samples were assayed for the presence of VSNJV antibodies using an IgM antibody capture enzyme-linked immunosorbent assay (gELISA), a competitive cELISA, and a serum neutralization (SN) test. Samples with a positive-over-negative ratio of ≥3.0 in the gELISA were considered positive for VSNJV antibody (Vernon and Webb 1985). A reduction value of ≥50% was indicative of VSNJV antibody in the cELISA (Katz et al. 1995). A reciprocal titer of >8 for a sample showing virus neutralizing activity in the microtitration format of the SN test was indicative of infection, but endpoint titers >512 were not determined because of their lack of diagnostic or predictive value (Snyder et al. 1982, Katz et al. 1997).

Serum concentrations of the acute phase proteins α-1-acid glycoprotein (AGP) and haptoglobin (Hp) were determined immediately before exposure to VSV and for 14 d thereafter. Commercially available radial immunodiffusion test kits (Cardiotech Services, Inc., Louisville, KY) were used (Morimatsu et al. 1992).

Insect Feeding Sites. The effect of insect feeding was assessed by allowing uninoculated biting midges to feed on steer 158 for 24 d before euthanization. Skin punch biopsies (Miltex Instrument Company, Inc., Lake Success, NY) containing papule-like lesions associated with insect feeding were collected 10 d postinsect exposure to attempt the isolation of potential infectious agents by the application of histopathology and electron microscopy analyses (O'Toole et al. 2003). These procedures were repeated with skin samples from the same area collected during necropsy.

Statistical Analyses. Data were analyzed using *t*-tests or two-way analysis of variance (ANOVA). Samples with unequal variances as determined by F analysis were analyzed using t' (Steele and Torrie 1980), or two-way ANOVA were performed. Transformed data (square-root transformation or square transformation) were analyzed for normality and equality of variance but did not show differences in significance compared with analyses of untransformed data.

Results

Feedings and Infection Rates of Insects. The numbers of *C. sonorensis* feeding on each steer are shown in Table 1. The success rates of blood feeding were significantly different among the steers for the inoculated insects ($\chi^2 = 166.4$, df = 3, $P < 0.001$), the uninoculated insects exposed after 24 h ($\chi^2 = 158.9$, df = 3, $P < 0.001$), and after 48 h ($\chi^2 = 239.3$, df = 3, $P < 0.001$). Four-hundred and forty-nine of 1,228 (36.6%) uninoculated biting midges blood fed on steer 158.

All of the intrathoracically inoculated *C. sonorensis* that completed a bloodmeal on a steer were tested for VSNJV infection (Table 2). There was no significant difference in the infection rates among the four groups of intrathoracically inoculated *C. sonorensis* that fed on the four steers ($\chi^2 = 1.9$, df = 3, $P > 0.5$). Individual infected biting midges contained $\approx 10^{2.5}$ $TCID_{50}$ VSNJV 10 d after intrathoracic inoculation.

Table 2. Infection rates of biting midges that took a blood meal from cattle

Steer no.	VSV inoculated biting midges			Uninoculated biting midges[a]			
	No. infected	No. uninfected	% infected	Time of feeding (h postinfection)	No. infected	No. uninfected	% infected
68	NA	NA	NA	24	0	33	0
				48	0	90	0
183	NA	NA	NA	24	0	150	0
				48	0	179	0
220	69	9	89	24	0	90	0
515	220	19	92	24	0	3	0
376	38	4	91	48	0	179	0
540	168	22	88	48	0	53	0

NA, not applicable.
[a] Biting midges uninfected at time of blood feeding were incubated for 10 d and then assayed for infection.

Table 3. Serologic response of cattle infected with VSNJ by intralingual injection in the tongue or through intrathoracically inoculated biting midges

Steer no.	Days postinfection																	
	0			4			6			8			14			22			39		
	SN	gELISA	cELISA	SN	gELISA	cELISA	SN	gELISA	cELISA	SN	gELISA	cELISA	SN	gELISA	cELISA	SN	gELISA	cELISA	SN	gELISA	cELISA
68*	<8	2.0	8	<8	3.8	30	256	15.6	69	>512	17.9	79	>512	17.6	86	>512	11	87	>512	4.7	89
183*	<8	1.6	18	<8	3.6	30	128	16.3	70	>512	18.8	83	>512	15.2	82	>512	7.4	87	>512	4.6	92
220	<8	2.7	5	<8	2.4	17	32	14.5	39	256	11.6	55	256	10.9	58	256	5.4	74	>512	3.2	65
515	<8	2.0	2	<8	1.9	10	256	13.8	65	>512	10.4	68	>512	7	70	>512	3.5	80	>512	2.2	88
376	<8	1.8	8	<8	2.3	20	64	11.7	48	256	8.7	62	>512	8.6	58	>512	4.7	60	>512	2.8	71
540	<8	2.5	18	<8	2.8	16	32	6.6	39	>512	10.6	62	>512	10.8	76	> 512	7	73	>512	4.5	86

For SN, numbers are reciprocal virus neutralization titers. For cELISA, numbers are percentage of reduction. For gELISA, numbers are ratio of positive over negative.

Clinical Observations. The steers intralingually inoculated with VSNJV developed vesicles at the inoculation sites in the tongue. However, differences were observed in vesicular stomatitis pathogenesis between the two animals. Grossly evident vesicles were detected in steer 68 at 24 h postinfection. The affected epithelium separated from the underlying tissues when the tongue was manipulated for closer inspection. Simultaneously, blanched slight elevations were observed at the sites of viral inoculation in the tongue of steer 183, which remained undisturbed upon manipulation. The area of vesiculation had enlarged in the tongue of steer 68 at 48 h postinfection, and sloughing of the epithelium was evident. The lingual lesions in steer 183 were ruptured at 72 h postinfection to collect affected epithelium. Infectious virus was isolated from oral swabs for up to 4 d postinfection. Steer 68 had a fever of 40°C 24 h after exposure, whereas the body temperature of steer 183 remained at <39.5°C until euthanization. Thus, gross clinical signs seemed more severe in steer 68.

None of the four steers infected with VSNJV via insect bite showed clinical signs. Infectious virus was not recovered from the oral swabs taken from these animals. The steers previously exposed to biting flies in the field showed signs of hypersensitivity at biting midge feeding sites. However, the steers that had been protected from biting fly attack developed a delayed onset papular dermatitis at insect feeding sites grossly similar to that observed in steer 158 (see below for details).

Application of Serodiagnostic Procedures. The serologic responses of cattle (Table 3) showed that the IgG antibodies differed significantly over time ($F = 58.14$; df = 6, 34; $P < 0.001$) and between intralingually inoculated steers and those exposed to insect transmission ($F = 25.84$; df = 1, 34; $P < 0.001$). IgM antibodies also showed differences between days and route of transmission ($F = 91.27$; df = 6, 34; $P < 0.001$ and $F = 28.14$; df = 1, 34; $P < 0.001$, respectively). Both steers inoculated in the tongue showed VSNJV IgG antibodies on day 4. On day 6, both steers also showed VSNJV antibodies by SN and cELISA. The insect-exposed steers all showed VSNJV antibodies by SN and gELISA on day 6, at which time one animal (515) also showed IgM antibody by cELISA. All animals had antibody present in all three tests by day 8. There were no significant differences between the insect-exposed and intralingually inoculated steers for the gELISA and cELISA values on days 0 and 39. The intralingually inoculated steers had significantly higher gELISA and cELISA values than the insect-exposed steers on day 4 ($t' = 6.8$, df = 4, $P < 0.01$), day 6 ($t' = 3.54$, df = 4, $P < 0.05$), day 8 ($t' = 5.78$, df = 4, $P < 0.0.01$), and day 22 ($t' = 3.62$, df = 4, $P < 0.05$), where t' represents t for samples with unequal variances. On day 14, the IgG gELISA values were significantly higher for the intralingually inoculated steers compared with the steers infected by insect bite ($t = 4.45$, df = 4, $P < 0.05$).

All animals maintained serum haptoglobin levels of less then 125 μg/ml throughout the experiment, and there were no significant differences in any of the

cattle. AGP levels were not significantly different over time ($F = 0.761$; df = 15, 64; $P > 0.05$) and were not different between inoculated and insect-exposed steers ($F = 1.19$; df = 1, 64; $P > 0.05$). Two steers showed a rise in AGP levels above the high normal levels of 450 μg/ml. Steer 515 had AGP levels of 470, 470, 470, 520, 560, 540, 420, 450, and 450 μg/ml on days 2–10 postexposure, respectively. Steer 540 had levels of 510, 430, 470, 430, and 470 μg/ml beginning on day 4 postexposure to day 8, respectively. Both of these animals also received VSNJV via *C. sonorensis* bite, and both animals had been exposed to biting fly activity under field conditions before being exposed to the virus.

Dermal lesions that were grossly similar to lesions reported at insect feeding sites on guinea pigs (O'Toole et al. 2003) were observed in steer 158 at biting midge feeding sites by 10 d postinsect exposure. The lesions were characterized microscopically by moderate multifocal superficial and interface dermatitis with serocellular crust formation and scattered multinucleated cells. The cattle lesions were more extensive and severe than the lesions previously observed in guinea pigs (O'Toole et al. 2003). At 24 d postinsect exposure, the lesions associated with insect feeding seemed microscopically as moderate multifocal subacute ulcerative and interface dermatitis with postnecrotic granulation tissue. Attempts to isolate an agent causing cytopathic effect, or to visualize a microbial agent by transmission electron microscopy, from skin samples of insect feeding sites at 10 and 24 d postinsect exposure failed.

Discussion

The role of biting flies in the epidemiology of vesicular stomatitis is poorly understood. Experimental data demonstrating susceptibility to infection in the laboratory and capability to transmit VSV have been shown for *S. vittatum* (Mead et al. 1999, 2000, 2004a,b). The observations reported here for *C. sonorensis* are similar to observations made for S. *vittatum.*

C. sonorensis has been infected with VSV through blood feeding on an artificial system in the laboratory (Nunamaker et al. 2000), and it has been shown to transmit VSV to guinea pigs during blood feeding (Perez de Leon et al. 2006). Our results show that intrathoracically infected *C. sonorensis* also transmit VSNJV to cattle, adding further support that this species should be considered a potential vector of VSNJV in the western United States.

The differences we observed in the blood-feeding success rates on the different cattle are typical when working with *C. variipennis* in the laboratory. The different groups of insects were exposed to the seven steers in four different rooms. We have observed that very subtle differences in factors such as light, humidity, and animal skin among others, may influence the feeding behavior of this species in the laboratory. No doubt, similar effects occur in the field, and this may influence the differences commonly observed between individual hosts in their attractiveness to biting fly activity. More work is needed on the role of these variables in the epidemiology of arthropod-borne animal pathogens such as VSV.

The steers inoculated with VSNJV directly in the tongue developed antibody earlier and had a greater initial antibody response then those animals receiving the virus through the bite of infected *C. sonorensis.* This is not surprising because the inoculum by syringe of 10^8 $TCID_{50}$ is probably greater than the amount of VSV delivered through *C. sonorensis* feeding and probably induces the earlier response because of the presentation of a greater amount of antigen. Because the intrathoracically inoculated *C. sonorensis* contained $\approx 10^{2.5}$ $TCID_{50}$ VSNJV per individual, the amount actually inoculated during feeding is substantially less, because less than 1 μl of saliva is probably delivered during blood feeding. Even if all insects fed on the steers, and their total viral load was completely inoculated into an animal, $\approx 10^4$ $TCID_{50}$ would be the maximum inoculum by this route. However, despite the smaller inoculum delivered by the infected insects, the antibody response was not significantly different from the syringe-inoculated animals at day 6.

VSNJV was only isolated by oral swabs from the intralingually inoculated animals, both of which developed oral lesions. Virus was not isolated from the blood or other tissues of any of the animals in this experiment. It is possible that the measured antibody responses of the animals were because of the initial inocula and not because of active viral replication. This was previously observed in guinea pigs exposed to infected *C. sonorensis* (Perez de Leon et al. 2006). We think the cattle were indeed infected with VSV, despite our inability to isolate live virus from the animals. Animals inoculated with VSNJV had active infections as evidenced by our ability to isolate VSNJV from oral swabs. Animals infected by insect bite received a much smaller challenge of virus, did not develop clinical signs, and produced antibody more slowly. The slow rise in antibody in these animals is consistent with an active VSV infection because it is unlikely that the small inocula delivered by insect bite would result in an antibody response that, although smaller, persisted for the same duration as the intralingually inoculated animals.

The possibility that animals infected by insect bite develop antibodies without active VSV infection or clinical signs raises interesting possibilities for understanding vesicular stomatitis epidemiology, as has been discussed for *S. vittatum* (Mead et al. 2004a,b). Serological surveillance of animal bloods has shown that ≈90% of VSV infections in livestock are subclinical infections during vesicular stomatitis epizootics in the western United States (Webb and Holbrook 1989). This is based on the assumption that animals with antibody are the result of active VSV infection and not because of antibody to initial viral challenge, as we discussed above. The origin and the effect of this large seropositive subclinical population and the role of arthropod transmission in the development of subclinical infection require further study.

Our findings support the interpretation of *S. vittatum* studies that subclinical infection is more likely through transmission by blood-feeding arthropods (Mead et al. 2004a,b), as we have observed in our study. The antibody response to the insect-transmitted virus was smaller than what might be observed through contact transmission of high-titer fluids, which may approach what is delivered through needle inoculation. If this is the case, the role of blood-feeding arthropods in the spread of VSV infection in the western United States may have far-reaching economic consequences. Animals with antibody but no productive infection are still considered a risk for transmission of VSV and are subject to restrictions in trade and movement (Tesh 1994). Additional studies are required to evaluate the possibility that insect-transmitted VSV may be a primary cause of subclinical vesicular stomatitis during vesicular stomatitis epidemics.

A significant acute-phase protein response for haptoglobin and AGP was not observed during our studies with VSNJV. This differs with the significant rise in haptoglobin levels observed at the onset of viremia and clinical signs in cattle infected with FMDV (Höfner et al. 1994). The failure of a response with FMDV in some animals was consistent with little tissue damage during initial foot-and-mouth disease (FMD) replication in the course of these experiments and that the virus replicated nonlytically, thereby escaping an acute-phase protein response. It also was suggested that the vesicles may not be the only stimulus for the rise in haptoglobins because there was a lag time between the appearance of FMD vesicles and haptoglobin response (Höfner et al. 1994). Both animals that experienced clinical signs (68 and 183) showed no significant increase in either acute-phase protein. Apparently, the level of viremia was not high enough to stimulate a response. Animals that showed high levels of AGP between days 2 and 8 (515 and 540) were exposed to heavy insect attack in the field before VSV exposure, indicating a possible systemic effect caused by hematophagous insect blood feeding (Tabachnick 2000). This warrants further investigation.

Phlebotomine sand flies did not become infected after feeding on domestic swine that had been inoculated with VSNJV intradermally (Comer et al. 1995). The failure to observe VSV transmission to *C. sonorensis* via blood feeding on cattle under laboratory conditions is consistent with the possibility that livestock may not be a predominant source for infecting insects in the natural cycle of VSNJV. If there is viremia in cattle (Hanson and Brandly 1957), it may be too low to infect large numbers of *C. sonorensis.* However, this deserves further study. There is great variation in the susceptibility of populations of arthropod vector species (Tabachnick 1994) such that there may be populations of *C. sonorensis* that are more susceptible to VSV infection via blood feeding than the colony of insects used in our studies. Thus, additional studies with other *C. sonorensis* populations are required. In addition, the dynamics of low host viremia and insect infection are complex such that hosts with low viremia can have a substantial role in transmission to vectors (Lord et al. 2006). Finally, it has been shown that *S. vittatum* can become infected with VSV through cofeeding with infected *S. vittatum* on nonviremic hosts (Mead et al. 2000), and mosquitoes can become infected with West Nile virus also through cofeeding on nonviremic hosts (Higgs et al. 2005). *C. sonorensis* may become infected in the field in this manner with substantial implications for understanding vesicular stomatitis epidemiology (Lord and Tabachnick 2002). This deserves further study.

It has been suggested that the effects of vector salivary factors on host defense mechanisms may play an important role in the transmission of VSV (Tabachnick 2000). Additional studies are needed to assess the potential for arthropods such as *C. sonorensis* to transmit VSV biologically and to better define the role of blood-feeding arthropods in the pathogenesis and epidemiology of vesicular stomatitis in the western United States. This information is expected to provide the basis to develop efficient control strategies to reduce the spread of VSV that will reduce the economic impact of vesicular stomatitis outbreaks on U.S. agriculture.

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